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## Properties of honey from *Tetragonisca angustula fiebrigi* and *Plebeia wittmanni* of Argentina\*

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**Abstract** – The composition of honey samples of *Plebeia wittmanni* ( $n = 10$ ) and *Tetragonisca angustula fiebrigi* ( $n = 12$ ) was analysed. The colours of all collected honeys were amber to dark amber and the pH varied. Moisture was lower than reported for other stingless bee honeys. Conductivity and ash content of *P. wittmanni* honey were higher than for *T. angustula fiebrigi*. Acidity of *P. wittmanni* honey was the highest ever mentioned for all other *Plebeia* species. Total sugars and sucrose were higher in *T. angustula fiebrigi* than in *P. wittmanni* honey. *T. angustula fiebrigi* honey showed the highest sucrose content ever mentioned and was rich in oligosaccharides. Both honeys split off sucrose,  $\alpha$ -glucosides, trehalose, and amylose. The strongest hydrolytic activity was on sucrose, with high activity for *T. angustula fiebrigi* honey. Raffinose was also hydrolyzed. The honey of both bees inhibited bacterial growth. These properties support, at least in part, the medicinal use of the stingless bee honey by native people.

***Tetragonisca angustula fiebrigi* / *Plebeia wittmanni* / Meliponini / composition / honey / antibacterial properties**

### 1. INTRODUCTION

More than 400–500 species of Neotropical stingless bees occur in the tropical and subtropical regions of America (Wille, 1979; Roubik, 1995; Camargo and Pedro, 2007). Beekeeping with stingless bees, or meliponiculture, was an aboriginal pre-Hispanic practice in Mesoamerica and their honey was a well known food resource of the aborigines in the North of Argentina (Alderete-Núñez, 1945). However, the introduction of *Apis mellifera* into the region produced a strong decline in meliponiculture. Different stingless bee species produce honeys with distinct organoleptic properties. Some of them

produce a fine, delicate honey with a delicious flavor (Kent, 1984; van Veen et al., 1990a) that allows their commercialization. The wax of these bees is used for waxing thread, covering cheeses, making candles (Kent, 1984). But traditionally, these honeys are not considered as food only. Mesoamerican aborigines use honeys as medicine for treating skin eruptions (confluent smallpox), tongue sores, urine problems, as emollient for wounds, cracked lips, skin infections and bruises, and treatment of upper respiratory system disorders. Diluted with water it is considered an ophthalmic remedy (Vit and Tomás-Barberán, 2004). Furthermore, it is used as purgative for women who have just given birth and as aid during birth. Because of the assigned properties, these honeys are sold to local pharmacies at a higher

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price than *A. mellifera* honey (van Veen et al., 1990b).

Several Meliponini species are recorded in the North of Argentina, and several are used as food and medicine. In Tucumán Province, Argentina, one may frequently encounter, even in the cities, a small black bee known as “pusquiyo” or “pusquillo” that was identified as *Plebeia wittmanni*. Another stingless bee from the same region with the common name “angelito” or “rubiecita” (=little blond) was identified as *Tetragonisca angustula fiebrigi*. Despite the frequent occurrence of these stingless bees, there are no Argentinean regulations for Meliponini honeys. Some regulations refer to *Apis mellifera* honey (Bianchi, 1990), and these are very similar to the European Directions and Regulations, or even more detailed, and in accordance with the *Codex Alimentarius*, International Honey Commission (CODEX 1969, 1987, 2001).

In the present study we report on colour, sugar composition, protein content, enzyme activities and antibacterial activity of honeys from *T. angustula fiebrigi* and *P. wittmanni* of San Miguel de Tucumán, Argentina.

## 2. MATERIALS AND METHODS

### 2.1. Honey sources

*P. wittmanni* ( $n = 10$ ) and *T. angustula fiebrigi* ( $n = 12$ ) honeys were collected around the City of San Miguel de Tucumán, (26° 48' 30" S 65° 13' 03" W) Argentina. The colonies were transferred from logs to wooden boxes of 20 × 20 × 10 cm in the case of *P. wittmanni*, and 30 × 30 × 30 cm for *T. angustula fiebrigi*, with removable bottom and lid parts. Honey sampled during December 2005 and January 2006 was taken from closed pots by suction with a syringe and immediately processed.

### 2.2. Physicochemical properties

#### 2.2.1. Colour

The colour of the samples was determined using the Lovibond comparator.

#### 2.2.2. Acidity and pH

Free acidity was determined by titration and pH was estimated using a digital pH meter Model HI 8519 (Hanna Instrument).

#### 2.2.3. Refraction index

The measurements were done with an Abbe refractometer (Atago, Japan), all taken at room temperature (26 °C). Before measurements, the refractometer's sample compartment and window were first cleaned with acetone.

#### 2.2.4. Electrical conductivity

The electrical conductivity was measured at 25 °C using a pH/Conductivity meter Model 20 (Denver Instrument). The instrument was calibrated using 0.01 M KCl.

#### 2.2.5. Moisture

For the determination of the moisture content, 2.50 g of each sample was placed in a flat dish and oven-dried at 105 °C for three hours, covered, cooled in a desiccator and weighed. The sample was then re-dried for one hour in the oven, cooled and reweighed. The process was repeated at one hour drying intervals until a constant weight was obtained. Values were expressed as moisture percentages. Each analysis was performed in triplicate.

#### 2.2.6. Ash content

For ash content determination, 2.50 g of each sample was put in a crucible and dried in an oven at 105 °C for three hours to prevent loss by foaming. After cooling it was ashed overnight in a Muffle oven at 600 °C. After cooling it was weighed to a constant weight.

### 2.3. Sugar determinations

#### 2.3.1. Mono and disaccharides

Total neutral sugars were determined by the phenol sulfuric acid method (Dubois et al., 1956) with

glucose as standard. Reducing sugars were determined by the Somogyi-Nelson method (Nelson, 1944; Somogyi, 1945) using glucose (Sigma-Aldrich, Saint Louis, Missouri, USA) as standard. Sucrose and fructose (Sigma-Aldrich, USA) were determined by the resorcinol method of Cardini et al. (1955) using sucrose as standard. Glucose was determined by the glucose-oxidase-peroxidase (Sigma-Aldrich, USA) coupled assay (Jorgensen and Andersen, 1973) with glucose as standard.

### 2.3.2. Oligosaccharides

A diluted honey sample (0.5 g of honey in 2 mL of distilled water) was adsorbed onto a 10 × 2.2 cm carbon-Celite column. The column was washed with 2 L of distilled water to eliminate soluble sugars and finally oligosaccharides were eluted with 70% ethanol (Merck, Damstadt, Germany). Fractions of 1.6 mL were collected and pooled. Oligosaccharides were determined as total sugars (Dubois et al., 1956).

### 2.4. Proteins

Proteins were determined by the method of Lowry et al. (1951) using bovine serum albumin (Sigma-Aldrich, USA) as standard.

### 2.5. Enzymes

#### 2.5.1. Enzyme preparation

Honey (10 g) was diluted with 3 mL of distilled water. Diluted honey was saturated (100%) with solid ammonium sulfate (Sigma-Aldrich, USA). After 30 min, the suspension was centrifuged at 20 800 g for 10 min, the precipitate was suspended in 1 mL of 10 mM sodium acetate buffer, pH 5.2, and the suspension was dialyzed against the same buffer (final Vol. 1.5 mL). One international enzyme unit (IEU) was defined as the amount of enzyme that produced 1 μmol of product in 1 min under defined conditions of pH and temperature.

#### 2.5.2. Enzyme assays

The incubation mixtures contained 10 μL of enzyme preparation, 40 μL of 0.2 M sodium acetate

buffer (pH 5.2), 40 μL distilled water, and 10 μL of 0.6 M sucrose or 50 μL of 0.6 M raffinose, trehalose or 10% amylase, respectively (Sigma-Aldrich, USA) in a final volume of 0.1 mL. The assays were performed at 37 °C for 30 min. Reactions were stopped by heating at 100 °C for 2 min.

The enzymatic hydrolysis and quantification of sugars was determined as reducing power release when sucrose or raffinose is used as substrate (Nelson, 1944; Somogyi, 1945); by the resorcinol method (Cardini et al., 1955), and/or by the specific measurement of glucose and fructose produced by the glucose-oxidase-peroxidase assay (Sigma-Aldrich, USA) (Jorgensen and Andersen, 1973), and by the enzymatic assay of D-fructose dehydrogenase (Sigma-Aldrich, USA), respectively (Ameyama, 1982). α-glucosidase activity was detected by reducing power release after enzyme extract was incubated with amylose as substrate.

### 2.6. Antibacterial activity

#### 2.6.1. Microorganisms

The microorganisms used included bacterial strains isolated from skin wounds at Hospital “Nicolás Avellaneda”, SIPROSA, Tucumán, Argentina. Gram negative bacteria: *Escherichia coli* (IEV301), *Pseudomonas aeruginosa* (IEV 305). Gram positive bacteria: *Staphylococcus aureus* (IEV7), *Staphylococcus aureus* coagulase-negative methicillin-sensible (IEV 20), *Enterococcus faecali* (IEV 208). Strains from American Type Culture Collection (ATCC) were also included; Gram negative bacteria: *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922). Gram positive bacteria: *Enterococcus faecali* (ATCC 29212), *Staphylococcus aureus* (ATCC 29213) and *Staphylococcus aureus* (ATCC 25923). Stock bacterial cultures were made in brain-heart infusion (BHI) medium (Britania S.A. Labs. Ciudad Autónoma de B. Aires, Argentina) supplemented with 0.3% w/v agar (Difco, Detroit, USA), 1.5% v/v glycerol (Sintorgan Labs., Vicente López, B.Aires, Argentina), and stored at -20 °C in sterile tubes. *Pseudomonas* cultures were kept in sterile water supplemented with 1% v/v glycerol at room temperature.

#### 2.6.2. Inoculum preparation

Stock bacterial cultures were kept at 37 °C for two hours. A loop of each strain was streaked onto

a Mueller-Hinton agar (solid Mueller-Hinton, Britania S.A. Labs. Ciudad Autónoma de B. Aires, Argentina) plate (10 cm diameter with 10 mL of medium), and incubated at 37 °C for 24 h. Each inoculum was prepared by suspending a colony from those plates in sterile water until it reached an optical density (OD) at 625 nm of  $0.09 \pm 0.01$ , which corresponds to  $10^8$  colony forming units (CFU)/mL (0.5 McFarland standard turbidity scale (Aiquel, 1977)).

### 2.6.3. Biological assays

An agar cup bioassay was employed for testing antibacterial activity of honey dilutions, following the standard procedure of Linday (1962). Honey dilutions were prepared by dissolving 10 g honey in 3 mL distilled water. The concentration of the resulting solution was 40% (w/v). Solid MH medium (10 mL) was poured into each Petri dish under aseptic conditions in a laminar flow chamber. When the medium in the plates solidified, 100 µL of the prepared inoculums were spread on each Petri plate with a sterile cotton swap. After inoculation, cups were scooped out with a 5 mm sterile cork borer and the lids of the dishes were replaced. Different quantities of sterile-filtered (Millipore filters, Millex-HV, 0.22 µm pore size) diluted honey (0, 10, 20 and 30 µL containing 0, 4, 8 and 12 mg of honey, respectively) were added to the wells and the volume completed to 40 µL with sterile water. Controls were made with a solution containing sucrose, glucose and fructose, at the same concentrations as those found in the honey dilutions to determine the effect of sugar concentration on microbial growth. Controls with penicillin G (for Gram-positive bacteria) and streptomycin (for Gram-negative bacteria) (Sigma-Aldrich, USA) were made. Controls for microbial growth were performed by adding the solvent (sterile water) to a well in each plate. Treatment and control samples were kept in an incubator at 37 °C for 24 h. The diameters of the inhibition zones were measured in four different directions; the mean and SD were calculated. Four replicates were made for each treatment.

### 2.7. Statistical analysis

Data were analyzed by One way Anova. Differences among means were determined using Tukey post hoc tests ( $P = 0.05$ ).

## 3. RESULTS

Physical parameters of the honey from *P. wittmanni* and *T. angustula fiebrigi* are summarized in Table I. The colour values for all of the *P. wittmanni* honey samples were around 128 to 140, and 80 to 105 for *T. angustula fiebrigi* honeys, corresponding to amber/dark amber on the Lovibond comparator scale range (from 60 to 250). All samples of *P. wittmanni* honey were darker than those of *T. angustula fiebrigi*. *P. wittmanni* honey was also more acidic than *T. angustula fiebrigi* honey ( $117.5 \pm 1.25$  and  $71.90 \pm 2.25$  meq/kg, respectively). The pH varied among the samples of *P. wittmanni* ( $2.0 \pm 0.1 - 4.0 \pm 0.15$ ) and *T. angustula fiebrigi* honeys ( $3.5 \pm 0.01 - 4.5 \pm 0.15$ ) with the variability of the latter being similar to that of *A. mellifera* (Siddiqui, 1970). The acidity of *P. wittmanni* honey was also higher than that of *T. angustula fiebrigi* ( $45.06 \pm 0.05 - 190.90 \pm 2.00$  and  $45.40 \pm 2.00 - 98.40 \pm 2.50$  meq/kg, respectively), in accordance with (Vit et al., 1994). Moisture of both types of honey was relatively low (Tab. I), viz.,  $12.35 \pm 1.70$  and  $17.05 \pm 1.85$  g/100 g, for *P. wittmanni* and *T. angustula fiebrigi* as measured by the oven method (Siddiqui, 1970; Vit et al., 1994). Total sugars and sucrose were high for the two bee species, with lower values for *T. angustula fiebrigi* than for *P. wittmanni* honey (Tab. I). In both honey types, fructose was twice the amount of glucose (Siddiqui, 1970). *T. angustula fiebrigi* honey appeared to be particularly rich in oligosaccharides (around 16% of the total sugars), whereas in *P. wittmanni* honey, oligosaccharides represented only about 7.5% of the total sugars. If considering that sucrose is comprised within the oligosaccharide content, then about 12.6% of the oligosaccharide content corresponds to unknown oligosaccharides for *T. angustula fiebrigi*, and to 4% for *P. wittmanni* honey. Both honeys are able to split off sucrose,  $\alpha$ -glucosides, trehalose, and amylose (Tab. II). The strongest hydrolytic activity of the two honeys was on sucrose, with *T. angustula fiebrigi* honey showing the highest activity. Raffinose, an oligosaccharide related to sucrose, was also hydrolyzed.

The antibacterial effects of diluted honeys from *T. angustula fiebrigi* and *P. wittmanni*

**Table I.** Physical properties and composition of the honeys from *Plebeia wittmanni* (n = 10) and *Tetragonisca angustula fiebrigi* (n = 12).

Determination	<i>Plebeia wittmanni</i>	<i>Tetragonisca angustula fiebrigi</i>
Colour (Lovibond units)	134 ± 11 <sup>a</sup>	92.5 ± 10 <sup>b</sup>
Range ± SD	128 ± 2 – 140 ± 2	80 ± 2 – 105 ± 1
pH	3.25 ± 0.25 <sup>a</sup>	4.25 ± 0.5 <sup>b</sup>
Range ± SD	2.0 ± 0.1 – 4.0 ± 0.15	3.5 ± 0.01 – 4.5 ± 0.15
Acidity (meq/kg)	117.5 ± 1.25 <sup>a</sup>	71.90 ± 2.25 <sup>b</sup>
Range ± SD	45.06 ± 0.50 – 190.90 ± 2.00	45.40 ± 2.00 – 98.40 ± 2.50
Refractive index	1.27 ± 0.10 <sup>c</sup>	1.47 ± 0.11 <sup>c</sup>
Range ± SD	0.75 ± 0.06 – 1.79 ± 0.09	0.98 ± 0.85 – 1.96 ± 0.95
Conductivity (µS/cm)	50.9 ± 2.0 <sup>a</sup>	28.2 ± 2.1 <sup>b</sup>
Range ± SD	39.80 ± 1.23 – 62.00 ± 0.98	17.47 ± 2.01 – 38.93 ± 1.95
Moisture content (g/100 g)	12.35 ± 1.70 <sup>a</sup>	17.05 ± 1.85 <sup>b</sup>
Range ± SD	9.35 ± 1.20 – 15.40 ± 2.10	11.10 ± 0.90 – 23.00 ± 1.25
Ash content by controlled heating (g/100 g)(Range ± SD)	0.16 ± 0.01 <sup>a</sup>	0.07 ± 0.01 <sup>b</sup>
Range ± SD	0.23 ± 0.02 – 0.09 ± 0.02	0.05 ± 0.01 – 0.09 ± 0.01
Total sugars (g/100 g)	72.71 ± 3.70 <sup>c</sup>	80.50 ± 7.50 <sup>c</sup>
Range ± SD	61.40 ± 1.60 – 84.02 ± 1.50	71.82 ± 0.90 – 89.19 ± 1.20
Reducing sugars (g/100 g)	69.55 ± 6.95 <sup>c</sup>	65.80 ± 4.60 <sup>c</sup>
Range ± SD	57.16 ± 0.90 – 81.94 ± 1.65	47.46 ± 1.25 – 84.24 ± 2.10
Sucrose (g/100 g)	4.01 ± 0.37 <sup>a</sup> ;	8.59 ± 0.67 <sup>b</sup>
Range ± SD	3.30 ± 0.80 – 5.85 ± 0.25	2.80 ± 9.98
Fructose (g/100 g)	45.05 ± 0.71 <sup>a</sup>	39.98 ± 1.80 <sup>b</sup>
Range ± SD	32.52 ± 1.50 – 57.52 ± 1.75	25.70 ± 1.02 – 54.26 ± 1.24
Glucose (g/100 g)	21.82 ± 0.20 <sup>c</sup>	22.00 ± 0.65 <sup>c</sup>
Range ± SD	16.00 ± 1.20 – 24.20 ± 1.65	17.05 ± 1.24 – 26.95 ± 1.85
Oligosaccharides (g/100 g)	7.45 ± 0.82 <sup>a</sup>	20.62 ± 1.05 <sup>b</sup>
Range ± SD	3.65 ± 0.65 – 11.25 ± 1.11	7.24 ± 1.30 – 34.00 ± 0.95
Proteins(g/100 g)	1.78 ± 0.32 <sup>c</sup>	2.02 ± 0.56
Range ± SD	1.40 ± 0.30 – 2.16 ± 0.45	1.10 ± 0.35 – 2.94 ± 0.55

Values are the minimum and maximum averages of each determination ± SD.

Different letters in the same row of the Table indicate significant differences between honey physical properties/composition of *Plebeia wittmanni* and *Tetragonisca angustula fiebrigi*.

**Table II.** Enzymatic activities in *Plebeia wittmanni* (n = 10) and *Tetragonisca angustula fiebrigi* (n = 12) honeys.

Substrate	<i>Plebeia wittmanni</i> (IEU*)	<i>Tetragonisca angustula fiebrigi</i> (IEU*)
Sucrose	4.40 ± 0.20	5.35 ± 0.50
Raffinose	0.85 ± 0.60	1.15 ± 0.50
Trehalose	0.16 ± 0.04	0.75 ± 0.09
Amylose	0.57 ± 0.07	0.77 ± 0.08

\* IEU: international enzyme units. Values are the mean of four determinations of each honey sample ± SD.

**Table III.** Antibacterial levels of diluted honeys (40%, w/v) from *Plebeia wittmanni* and *Tetragonisca angustula fiebrigi* in 10<sup>8</sup> CFU/mL.

Microorganism	Honey*				Control
	<i>P. wittmanni</i> (µL)		<i>T. angustula fiebrigi</i> (µL)		
	0	10 20 30	0	10 20 30	
Gram negative bacteria					Penicillin-G (30 µg /mL)
<i>E. coli</i> (ATCC 25922)	--	6 9	---	7	12
<i>E. coli</i> (IEV301)	--	6 9	---	7	15
<i>P. aeruginosa</i> (IEV 305)	--	7 9	----		20
<i>P. aeruginosa</i> (ATCC 27853)	--	7 10	---	6	18
Gram positive bacteria					Streptomycin (30 µg/mL)
<i>S. aureus</i> (IEV7)	--	7 10	--	6 8	14
<i>S. aureus</i> coagulase-negative methicillin-sensitive (IEV 20)	--	7 10	--	6 8	12
<i>E. faecali</i> (IEV 208)	--	7 10	----		13
<i>E. faecal</i> (ATCC 29212)	--	7 11	----		12
<i>S. aureus</i> (ATCC 29213)	--	7 11	--	6 8	13
<i>S. aureus</i> (ATCC 25923)	--	7 10	--	6 8	13

\* µL of the dilution of 10 g of honey in 3 mL water.

-- : No action on bacterial growth.

Bacterial growth was not inhibited by glucose, fructose and sucrose assayed at the same concentrations found in honeys. These sugars were assayed separately in different experiments and together.

on several pathogenic bacteria are shown in for Table III. The honey of both bees were effective against bacteria, however, this effect was less so in the case of *T. angustula fiebrigi* honey. A noteworthy difference was found in the case of two *P. aeruginosa* strains, where one strain (*P. aeruginosa* ATCC 27853) was susceptible to *T. angustula fiebrigi* honey, whereas *P. aeruginosa* IEV 305 showed resistance to the same honey. Moreover, the growth of the two strains of *E. faecalis* assayed was inhibited by *P. wittmanni* honey, but not by that of *T. angustula fiebrigi*. Growth controls performed in a separate experiment with sucrose, glucose and fructose kept at the same concentrations found in honeys, well as with the three sugars together showed that the sugar concentration alone does not affect bacterial growth. Consequently, the antibacterial activity of these honeys is likely due to non sugar components.

#### 4. DISCUSSION

Stingless bee honeys were a well known food and medicine of the aborigines in the

Northwestern Argentina. In terms of dietary aspects these honeys compared well with the honey of *A. mellifera*, according to the present results, Moreover, the peasants of the North of Argentina attribute medicinal properties to these honeys, and they are. used as eye drops against eye irritations, and also against cataracts (Vit et al., 2004), ocular ulcers, for cicatrization of wounds, upper respiratory tract diseases, and against skin infections.

The bacteria assayed in this paper were clinical isolates obtained from public Hospitals and have ample incidence in northwestern Argentina. They show variable resistance to antibiotics (Soberón et al., 2007). Furthermore we assayed strains from the american type culture collection (ATCC) that were selected as reference for the behavior of Gram-positive and Gram-negative bacteria. The enterobacteria present in the intestinal track have an important prevalence as infective agents in other parts of the body, such as skin, from which they were isolated. *S. aureus*, a methicillin-resistant Gram positive strain isolated from skin, worldwide produces serious intranosocomial infections that are difficult to eradicate (Vandenesch et al., 2003).

The antibacterial effect of *T. angustula fiebrigi* honey from northwestern Argentina was similar to that of *T. fiebrigi* honey from Paraguay and northeastern Argentina (de Almeida et al., 2009; Rodriguez-Malaver et al., 2009; Vit et al., 2009), and to *T. angustula* honey from different regions of Costa Rica (DeMera and Angert, 2004). In all these Gram-negative bacteria were the most resistant to honey treatment. Moreover, bacterial growth inhibition by these honeys was better than that found for other species of stingless bees of Guatemala (Dardón and Enriquez, 2008). The results of the antimicrobial screening, thus, qualify the traditional use of these honeys for topical treatments in emergency situations when commercial antibiotics are not available. What is also important is that this reinforces the concept that investigations of the traditional usage of natural products will reveal a substantial number of positive responses in in vitro screens. Furthermore, as shown by sugar concentration controls, the antibacterial activity of these honeys is likely due to non-sugar components (Vit and Tomás-Barberán, 2004). Since the antibacterial activity of stingless bee honey is still poorly studied, some authors propose that this antibacterial activity may be ascribed to a contamination with propolis (Dardón and Enriquez, 2008).

With respect to physical properties, both stingless bee honeys studied herein showed some variation that can be ascribed to characteristics of the bees' physiology, their preferences for certain flowers, or to differences in honey processing by the two bee species.

The dark amber colour of the honeys was ascribed to a higher mineral content than in the lighter ones (White, 1975). This is in accordance with a higher ash content in *P. wittmanni* honeys and an elevated conductivity. Both honeys are acidic and similar to stingless bee honeys from Venezuela (Vit et al., 1994; de Almeida et al., 2009). In contrast, the marked differences as regards moisture content may likely be species-specific.

Considering the lack of hydrolysis of  $\alpha$ -galactosides by both honeys, raffinose is probably hydrolyzed by a honey  $\alpha$ -glucosidase. The presence of  $\alpha$ -glucosidase activity in honeys was confirmed by the attack on

trehalose and  $\alpha$ -methyl glucoside. Moreover, both Meliponini honeys contain more sucrose ( $3.66 \pm 0.37$  and  $8.59 \pm 0.67$  g/100 g honey) than *A. mellifera* honey ( $1.5$ – $2.1$  g/100 g honey) (Tourn et al., 1980) which appears strange considering their high sucrose splitting activity. It may be possible that the reaction products themselves, fructose and glucose, are inhibitors of the enzyme for further sucrose hydrolysis.

The composition of stingless bee honeys has been studied far less than that of *Apis mellifera* honey, and consequently, standards for quality control of Meliponini honeys are not yet established in the countries where they are produced (Gonnet et al., 1964; Cortopassi-Laurino and Gelli, 1991; Vit et al., 1994, 2004; Vit and Pulcini, 1996; Souza et al., 2006). In this respect, the present study is the first contribution reporting the composition of *P. wittmanni* honeys, and extends the information given in previous reports on *T. angustula fiebrigi* honey (Souza et al., 2006; de Almeida et al., 2009; Rodriguez-Malaver et al., 2009; Vit et al., 2009) of northwestern Argentina. Furthermore, the results on antibacterial effects may explain their frequent and ample usage in Traditional Medicine.

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**Propriétés du miel produit par *Tetragonisca angustula fiebrigi* et *Plebeia wittmanni* en Argentine.**

**abeilles sans aiguillon / *Tetragonisca angustula fiebrigi* / *Plebeia wittmanni* / Meliponini / composition du miel / propriétés antibactériennes**

**Zusammenfassung – Die Eigenschaften von argentinischen *Tetragonisca angustula fiebrigi*- und *Plebeia wittmanni*-Honigen.** Stachellose Bienen sind einheimische Bienen der tropischen und subtropischen Zonen Amerikas und wurden von Einheimischen als Honigquelle und als Medizin benutzt. Von Zentralamerika bis ins tropische und subtropische Südamerika fanden ihre Honige Verwendung in der Behandlung von Infektionen der Augen, Wunden und des oberen Atmungstrakts, sowie als Abführmittel. Die Quechua-Sprache unterscheidet verschiedene Stachellose Bienen-Arten Nordwest-Argentiniens als *tiu-simi*, *yana*, *kayasan*, *kella* und *pusquillo*. Die physikalischen Parameter und die Zusammensetzung von Honig der Arten *Trigona (Tetragonisca) angustula fiebrigi* und *Plebeia wittmanni*, die alle in der Provinz Tucumán, Argentinien gewonnen wurden, sind in Tabelle I zusammengestellt. Die Analyse der Zucker zeigte, dass Glukose und Saccharose die Hauptkomponenten bilden. Hinsichtlich der enzymatischen Aktivität dieser Honie konnten wir zeigen, dass sie in der Lage sind, Saccharose,  $\alpha$ -Glykoside, Trehalose und Amylose aufzuspalten. Die stärkste hydrolytische Aktivität fanden wir bei zwei *T. angustula fiebrigi*-Honigen, wobei diese gegen Saccharose gerichtet war. Raffinose, ein Saccharose-ähnliches Oligosaccharid wurde ebenfalls hydrolysiert (Tab. II). Verdünnte Honige von *T. angustula fiebrigi* ( $> 30 \mu\text{L}$ ; 30 % w/v) und *P. wittmanni* ( $> 20 \mu\text{L}$ ; 20 % w/v) zeigten antibiologische Eigenschaften gegen verschiedene pathogene Bakterien (*Escherichia coli*, *Pseudomonas aeruginosa*, *Enterococcus faecalis* und *Staphylococcus aureus*) mit einer Konzentration von  $10^8$  CFU/mL (Tab. III). Diese Eigenschaften erklären die medizinische Verwendung der Honige Stachelloser Bienen in ihren Ursprungsländern, v.a. durch die einheimische Bevölkerung.

***Tetragonisca angustula fiebrigi* / *Plebeia wittmanni* / Meliponini / Zusammensetzung / Honig / antibakterielle Eigenschaften**

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